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► **To cite this version:**

Georgia M. Kefala, Daria Tsvirkun, Claude Verdier, Valérie M. Laurent. Changes in endothelial cell microrheology in medium modified by cancer cells. *Computer Methods in Biomechanics and Biomedical Engineering*, 2023, 48eme Congrès de la société de biomécanique, 26 (S1), pp.S43-S45. 10.1080/10255842.2023.2246304 . hal-04061684v2

HAL Id: hal-04061684

<https://hal.science/hal-04061684v2>

Submitted on 29 Jun 2023

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Changes in endothelial cell microrheology in medium modified by cancer cells

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1. Introduction

During the process of metastasis, cancer cells are detached from the primary tumour and may find their way into the blood system, from where they can migrate in a distant site. Endothelial cells line the vascular wall of all blood and lymphatic vessels and regulate its permeability for substances and migratory cells. Previous studies have focused on the properties of cancer cells (Abidine *et al.* 2018), as well as their interaction with endothelial cells (Rajan *et al.* 2016), as they attach to and transmigrate through the endothelium barrier. For many authors, the most widely accepted hypothesis was that transmigration is a characteristic of cancer cells, overlooking the possibility of endothelial cells actively participating in this process (Mierke *et al.* 2012). In this paper, endothelial monolayers were studied with a previously developed AFM microrheology technique (Abidine *et al.* 2018) when cultured in cancer cell conditioned medium, at different dilutions. This medium is enriched with molecules secreted by the cancer cells, which may play a role during the cancer cells' migration and alter the endothelial cells' behaviour (Ritchie *et al.* 2021). We show the first results of the viscoelastic properties of endothelial cells under these experimental conditions.

2. Methods

2.1 Cell culture and preparation

Human Umbilical Vein Endothelial Cells (HUVECs) were cultured in supplemented Endothelial Cell Growth Medium (ECGM, PromoCell) at 37°C, 5% CO₂, 95% humidity. Cells with passage numbers 4-6 were used. T24 cells (Human bladder carcinoma cells) were grown in RPMI 1640 medium (Gibco) supplemented with 10% Fetal Bovine Serum (Gibco) and 1% penicillin-streptomycin. For the AFM measurements, HUVECs were seeded in monolayer in Petri dishes with glass coverslips at the bottom, at least 72 hours before the experiment. Less than 24 hours before the measurements, the medium was exchanged with a mixture of ECGM and T24-

conditioned ECGM (ECGM-T24) in ratio 2:3 (low dilution) and 1:10 (high dilution) correspondingly. ECGM-T24 was prepared by culturing T24 cells in ECGM for 2 days.

2.2 Microrheology

We used a novel AFM technique which allows for a microrheological study of cells, by applying an initial indentation δ_0 and superposing a small oscillatory displacement δ . The elastic modulus E can be calculated from the microrheology data during the initial approach, by using the Hertz formula

$$F_0 = \frac{3E \tan \theta \delta_0^2}{4(1-\nu^2)} g[\chi(\delta_0, h)], \quad (1)$$

where $F_0 = kd$ is the initial force, k is the cantilever stiffness and d its vertical displacement. ν is the cell's Poisson's ratio (~ 0.5), $\theta = 19^\circ$ the half angle of the pyramid cantilever tip, and $g[\chi(\delta_0, h)]$ a correction factor for the hard substrate effect, dependent on the parameter $\chi = \frac{\delta_0}{h} \tan \theta$, with h the height of the cell (Abidine *et al.* 2018). The elastic modulus G' and viscous modulus G'' can be calculated as a function of frequency using the linearized form of (1) (Alcaraz *et al.* 2003). Cantilevers with a pyramidal tip and with stiffness approximately 0.016 N/m were chosen. An approach set point of 0.5 nN and a displacement of $\delta/\delta_0 = 0.1$ were used for the measurements. A range of oscillatory frequencies between 1Hz and 160Hz was used. The indentation was always performed on the top of the nucleus. The statistical significance was tested with unpaired t-test and Wilcoxon's test. The results are displayed as Mean \pm SEM (Standard Error of the Mean).

3. Results and discussion

AFM measurements were performed on single cells in endothelial monolayers. The indentations on the cells were in the range of 0.4-1.2 μm . Measurements were taken on endothelial cells cultured in conventional ECGM, serving as the controls ($n=8$, $n=20$), on endothelial cells cultured in T24-conditioned ECGM in low ($n=26$) and high dilution ($n=20$). Height measurements on HUVECs cultured in T24 medium (low: $2.6 \pm 0.2 \mu\text{m}$, high: $2.4 \pm 0.2 \mu\text{m}$) did not show significant differences from the controls (low: $2.7 \pm 0.3 \mu\text{m}$, high: $2.5 \pm 0.3 \mu\text{m}$). In Figure 1, the Young's modulus E as calculated using equation (1), is displayed. For both dilutions, the endothelial cells cultured in ECGM-T24 showed lower E values (low: $4.4 \pm 0.9 \text{ kPa}$, high: $1.5 \pm 0.3 \text{ kPa}$) compared to their controls (low: $6.9 \pm 2.3 \text{ kPa}$, high: $2.4 \pm 0.4 \text{ kPa}$). This indicates that T24 secreted molecules in the medium influenced the HUVECs, by decreasing their stiffness. The p values were bigger than 0.05, denoting the need of a larger amount of data to reach significance.

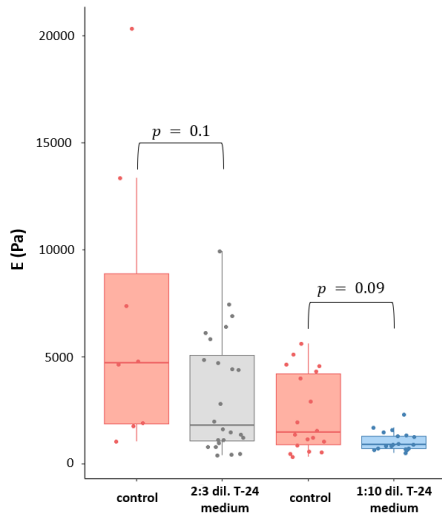


Figure 1 Young's modulus E of controls (red, $n=8$ and $n=20$), HUVECs in 2:3 (grey, $n=26$) and 1:10 (blue, $n=20$) diluted ECGM-T24.

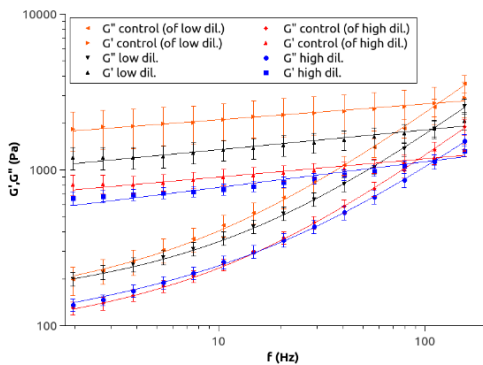


Figure 2 Moduli $G'(f)$ and $G''(f)$ as a function of frequency for HUVECs cultured in (a) high and (b) low dilution. The data displayed are averaged over $n=26$ for low and $n=20$ cells for high dilution, as well as $n=8$ and $n=20$ for their controls respectively. The error bars correspond to SEM.

Figure 2 shows the shear moduli G' and G'' , as a function of frequency. The moduli of the 2:3 and 1:10 dilution experiments are displayed in black and blue, respectively. In orange and red, the curves of the corresponding controls are shown. The fitting was performed following the model of Alcaraz *et al.* (2003), where $G' = af^b$ and $G'' = cf^b + df$. The slope b was found to be the same for controls and HUVECs in 2:3 dilution ECGM-T24 ($b=0.1$) and similar for the 1:10 dilution case ($b_{con}=0.1$, $b=0.16$). For both experiments, the control G' values were higher than those of HUVECs in T24-ECGM. The ratios $r_{G'} = G'_{control}/G'$ showed that the high dilution case was closer to the control curve ($r_{G'}=1.1$, $f=20\text{Hz}$) than the low one ($r_{G'}=1.5$, $f=20\text{Hz}$). HUVECs became softer with decreasing the dilution of ECGM-T24. Previous studies on endothelial cells co-cultured with cancer cells also showed a decrease in their stiffness

(Mierke *et al.* 2011). Based on our findings, culturing HUVECs in cancer cell medium was sufficient to observe differences in their microrheology. Nevertheless, more experiments are needed, including fluorescence imaging to analyze the cytoskeleton.

4. Conclusions

Elastic and microrheological measurements showed that endothelial cells were softer when cultured in cancer cell conditioned medium. Our results of the elastic moduli E and G' showed that the more concentrated (secreted substances) the medium was, the softer HUVECs became. In the future, these findings will be compared with the co-culture of HUVECs and T24 cells, to better assess the impact of cancer cell-secreted molecules and the physical attachment of cancer cells on the alteration of endothelial cell properties.

Acknowledgements

V.M.L. and C.V. are members of the LabeX Tec 21, France (Investissements d'Avenir: grant agreement No. ANR-11-LABX-0030).

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Keywords: endothelial cells; viscoelasticity; AFM; cancer cell transmigration; microrheology;

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